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Fusion of human erythrocyte ghost membranes was studied by using FITC-dextran and 1,1'-dihexadecyl-3,3,3',3'-tetramethylindo carbocyanine perchlorate (DiI) to detect, respectively, individual contents mixing events and membrane mixing events in populations of human erythrocytes. An electric field pulse was used as a fusogen and dielectrophoresis was used to reversibly induce membrane-membrane contact. We found during the first year of this project that when human erythrocyte ghosts in 20 mM or 60 mM sodium phosphate (pH 8.5) are treated with an appropriate electric field pulse to induce membrane fusion, both fusion-associated contents mixing and nonfusion contents mixing are observed [Sowers, A.E. (1988) Biophys. J. 54, 619-626]. In the present study separate assays conducted on the same membrane preparation under identical conditions suggest that: i) the nonfusion contents mixing events are an artifact due to electroporation, ii) at 20 mM, fusion-associated contents mixing events (calculated by substracting the fraction of nonfusion events) compare favorably with membrane mixing

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events over a very wide range of fusion yields, iii) at 60 mM the nonfusion contents mixing events are suppressed, but interfering processes cause the fusion-associated contents mixing events to not compare favorably with membrane mixing events, and iv) electropores are not likely to be a fusion intermediate in the fusion mechanism.

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INTRODUCTION

Our work to elucidate the mechanism of membrane electrofusion utilized dielectrophoresis (Pohl, 1978) to induce close membrane-membrane contact. This method is convenient, mild, completely reversible, and nonchemical. Also, A single electric field

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Notes:

1. Abbreviations: CM, contents mixing; Da, dalton; DiI, 1,1',-dihexadecyl-3,3,3',3'-tetra-methylindo carbocyanine perchlorate; E, pulse electric field strength; Q, pulse energy; FD, FITC-dextran; FITC, Fluorescein isothiocyanate; FY_{mm}, fusion yield based on membrane mixing events; FY, fusion yield; FY_{cm}, fusion yield based on contents mixing; FY_{cm+a}, fusion yield based on contents mixing and attachment; MM, membrane mixing; mM, millimolar; msec, millisec.; N_m, number of labeled membranes showing indicator mixing; N_o, number of labeled membranes not showing indicator mixing; S, buffer strength; $T_{1/2}$, decay half-time; V_{rms}/mm, root-mean-square volts per millimeter.

2. Definitions: A FY which is calculated from CM or MM data will be designated FY_{cm} or FY_{mm}, respectively. In our work, the observation of permanent attachment (=fusion) between two membranes in which contents mixing took place was found to be the minimum criteria for fusion. In this case, the fusion yield was designated FY_{cm+a}.

3 - Acknowledgement: The technical assistance of V. Kapoor is acknowledged.

pulse was used as a fusogen because it permits fusion events to be induced at will and fusion yields can be high enough to make practicable the observation of single events. Moreover, the high time resolution permits data recording to be coordinated with the initiation of fusion. This combined approach, permits membrane-membrane contact, the fusogen, and the chemical conditions in the medium to be manipulated independently of one another. This represents a fundamentally new approach to the study of membrane fusion. Our studies have revealed a number of significant new details about the membrane fusion process and how to make valid measurements of fusion yield.

Electric pulse-induced fusion in erythrocyte ghosts in 20 mM sodium phosphate buffer (pH = 8.5) leads to two categories of fusion product. In the first case, the fusion product is visible by light microscopy which uses phase contrast optics (Fig. 4 in Sowers, 1984). In this case the diameter of the hour-glass constriction (lumen) reaches 3 - 7 um within 10 sec after the pulse (Sowers, 1985) and is clearly visible by phase optics. In the second case, the fusion product can only be detected indirectly by light microscopy using phase optics (Fig 6 in Sowers, 1984). This is because the lumen diameter remains small enough to be submicroscopic. The following experimental observations indicate that these nonlumen-producing fusion products are true fusions. First, the fluorescent lipid analog, DiI, used to label a fraction of the ghosts in the pearl chains will laterally diffuse from originally labeled membranes to one or more adjacent but originally unlabeled ghost membranes if and only if a fusogenic electric field pulse is applied to the membranes (Fig 5b-e in Sowers, 1984). Second, release of membranes in pearl chains from the dielectrophoretic force after the pulse showed by phase optics that a fraction of all of the ghosts in the pearl chains became irreversibly attached to each other in linear polysphere groups. These groups contained 2-4 ghosts each while the remainder of the ghosts returned to random positions (Fig. 6 in Sowers, 1984; Sowers, 1988). This is consistent with the conclusion that two ghost membranes are connected to each other by a submicroscopic lumen. Third, if the above linear polysphere groups contained a mixture of DiI-labeled and unlabeled ghosts and were examined by fluorescence optics, then 99% of the linear polysphere groups also showed fluorescence laterally diffusing into at least one originally unlabeled membranes until equilibrium in fluorescence was achieved (Sowers, 1988). In other words, if irreversible attachment was observed between ghost membranes, then lateral diffusion was also observed and vice versa. This observation confirmed that all fluorescence-bearing membranes shared membrane continuity. This implies that there was a one-to-one correspondence between either attachment (representing a physical connection) or lateral diffusion of DiI (a membrane mixing event) and a fusion event. The above observations have been independently observed in another laboratory (Ahkong and Lucy, 1986; Lucy and Ahkong, 1988).

Other experiments showed that the relative numbers of lumen-

producing and nonlumen-producing fusions can be controlled independently of fusion yield by adjusting the concentration of glycerol in the buffer (Sowers, 1984). Also, of all fusions the fraction which is lumen-producing is approximately proportional to the strength of the sodium phosphate buffer between 20 and 60 mM (Sowers, unpublished). However, at or below 15 mM, lumen-producing fusions never occur (the question of whether the lumen inside diameter in nonlumen fusion products is large enough to pass CM indicator molecules is addressed below)(Sowers, 1989).

Conceptually, fusion of two membranes should result in a single membrane which encloses a single space (Gingel & Ginsberg, 1978). Thus it should be possible to experimentally demonstrate for each fusion event both a membrane-mixing (MM) event and a contents-mixing (CM) event. This is important because agreement between these assay results would indicate that the assays are valid. While our earlier studies showed that both individual CM events and MM events were easily detectable (Sowers, 1984), we subsequently observed a large discrepancy between CM and MM events when we conducted separate but identical fusion assays using DiI and FD as mixing indicators on the same ghost preparation (Sowers, 1988). In most cases FY_{cm} exceeded FY_{mm} by a margin well above experimental error. In the same study, however, a modification in the experimental protocol using a different ghost preparation made it possible to determine that a fraction of all of the contents mixing events actually represented an artifact rather than membrane fusion (see Fig. 1).

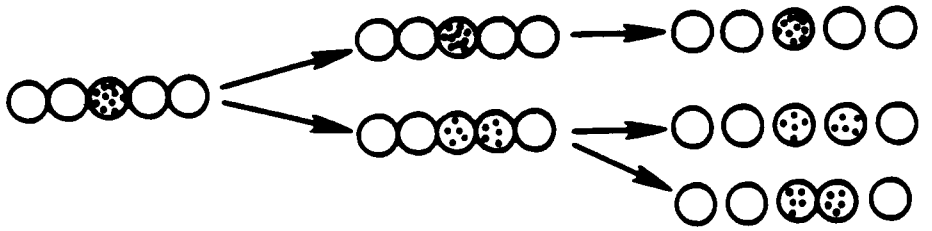

Fig. 1. Protocol for contents mixing assay. Left: alignment of unlabeled erythrocyte ghosts and erythrocyte ghosts containing mw = 10 kDa FITC-dextran into a pearl chain by low strength alternating current-induced dielectrophoresis. Originally unlabeled membranes are visible by phase optics. Unlabeled membranes which becomes labeled by FD (dots) after the pulse are visualised by fluorescence optics. Middle: two fates of aligned ghosts when a fusion-inducing pulse is applied. Upper: no contents mixing, Lower: contents mixing between two (shown) or more (not shown) adjacent ghosts as fluorescence (dots) moves to right (cf. Left). Right: fates of aligned ghosts after cessation of dielectrophoresis and lapse of sufficient time for Bownian Motion to separate unfused membranes. Upper- all membranes separate as a result of Brownian motion. Middle- all membranes separate as result of Brownian motion despite a contents mixing event. Lower- only fused membranes which show contents mixing remain attached (by a lumen) to each other and do not separate.

The modification in the protocol was composed of a step in which: i) the dielectrophoretic force was removed after the fusion-inducing pulse, and ii) allowing enough time for Brownian motion to cause those membrane groups which showed contents mixing but did not fuse to eventually separate from one another by enough distance to be visually observable. This modification in the protocol made it possible to clearly reveal which contents mixing events actually represented fusion events. However, the fact that a different ghost preparation was used was what prevented a quantitative correction to be performed and a quantitative comparison made. Despite this shortcomming, that study (Sowers, 1988) detected and demonstrated the principle of the nonfusion contents mixing artifact.

A separate experiment further clarified the relationship between contents-mixing events and membrane fusion. In this experiment pearl chains containing mixtures of FD-labeled and unlabeled ghosts were treated with a pulse and then only the ghosts irreversibly attached to each other (=fusion) in linear polyspheres were located by phase optics after the AC was turned off. When these polysphere groups of ghosts were examined by fluorescence optics the FD was always (99 %) found, independent of fusion yield, in at least two adjacent ghosts (Sowers, 1988). In the absence of a fusion-inducing pulse, all (98-99%) of the fluorescent ghost membranes in pearl chains were found as single entities. This showed that every pulse-induced attachment event (= fusion) was also accompanied by a contents-mixing event, but, conversely, every contents-mixing event, by itself, did not demonstrate a fusion event unless attachment could be demonstrated after the step in which the membranes were released from the dielectrophoretic force (Fig. 1).

Present work. The present work addresses the hypotheses that: i) a valid FY based on a contents mixing assay can be derived by subtracting from all CM events those CM events which are not due to fusion, and ii) such a FY should agree with a FY derived from the use of a membrane mixing assay. Table I shows FY_{mm}, FY_{cm}, and FY_{cm+a} in the same ghost preparations as a function of pulse field strength, E, pulse decay half-time, $T_{1/2}$, and buffer strength, S, as the experimental variables. FY_{mm}, FY_{cm}, and FY_{cm+a} were all proportional to pulse E and $T_{1/2}$, which is consistent with that observed previously (Sowers, 1988) and the membrane electrofusion literature in general. While changing buffer strength from 20 mM to 60 mM produced a small increase in FY_{mm} and a small decrease in FY_{cm+a}, it also produced a large decrease in FY_{cm}. These trends were also in agreement with previous observations (Fig. 3 in Sowers, 1988).

Two interrelated conclusions can be drawn from the present study. First, at S = 20 mM, the two fusion assays essentially agree with each other over a wide range of fusion yields (7% < FY_{mm} < 72%). Although a large fraction of all CM events are artifactual (nonfusion), an essentially correct FY based on CM events can be derived by a straightforward determination of

FY_{cm+a} instead of using FY_{cm}. Second, the relative number of artifactual nonfusion contents-mixing events are much lower at S = 60 mM. However, there is a very rapid increase in the pulse strength-dependent discrepancy between FY_{mm} and FY_{cm+a} and membrane fragmentation takes place above a sharp threshold in Q (Fig. 2). Both of these observations indicate interference to the fusion process by effects which may be irrelevant to fusion. It is also possible, however, that during the formation of the fusion product the reduced FY_{cm+a} observed at 60 mM is due to a lumen diameter which is too small to pass contents mixing indicators (see below).

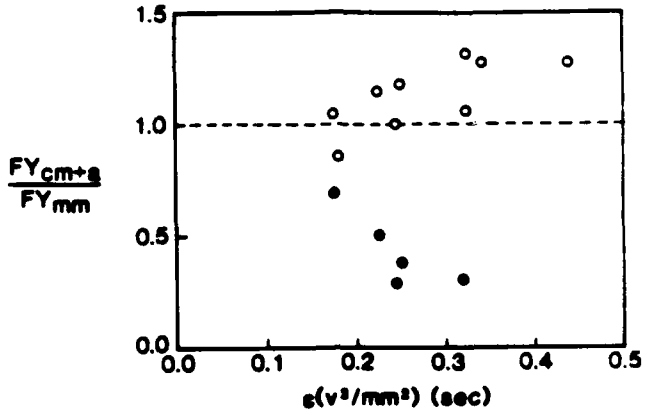

Fig. 2. Ratio of FY_{cm+a} to FY_{mm} as a function of pulse energy Q = $(E^2)(T_{1/2})$ in (kV^2 msec). Buffer strength: open circles, 20 mM; solid circles, 60 mM. At 60 mM fragmentation occurred at Q > 0.32 (see text). Observation: At a buffer strength of 20 mM FY_{cm+a} was close to FY_{mm}. In the 60 mM buffer the ghost membranes fragmented to small vesicles for pulses in which the product of pulse field strength (E) and pulse decay half-time $(T_{1/2})$, was above a threshold defined by $Q = (E)(T_{1/2}) > 0.32$ (in kV^2msec) (Fig. 2). In specific, when $Q < 0.32$, FY_{cm+a} was within 8 % (ave) of FY_{mm}. But when $Q > 0.32$, then FY_{cm+a} was within 23 % of FY_{mm}. However, at a buffer strength of 60 mM the FY_{cm+a} was much less than FY_{mm}. For example, at E = 500 V/mm and $T_{1/2}$ = 0.7 the FY_{cm+a} was no closer than 33 % of FY_{mm}, and more than 50 % from FY_{mm} for all other pulse field strengths and decay half-times.

Incorporation of the present data and previous observations into a hypothetical explanation requires that two other known factors be taken into account. First, electropores (for reviews see Tsong, 1983; Neumann et al, 1982; Knight & Scrutton, 1986) are also induced by electric field pulses having about the same strength and duration characteristics as pulses which will induce membrane fusion. While little solid information exists about numbers, effective diameters, and locations of electropores in the membranes, their induction appears to be very rapid (Tsong, 1983) compared to their resealing time (Chernomordik et al.,

1987; Schwister & Deuticke, 1985; Serpersu et al., 1985). Second, there is good experimental evidence that the passage of the contents-mixing indicators through electropores may be driven by electroosmosis (Sowers, 1988). This causes a preferential movement of CM indicators towards the negative electrode during CM events.

Figure 3 shows both electropore induction and electrically-induced fusion as two independent but self-completing (irreversible) processes and the effect of these processes on a CM indicator contained in one of two adjacent ghost membranes held in a pearl chain. Thus a CM event could occur either by a fusion process (Path 1) or by virtue of an artifact (path 2) and therefore not be related to fusion. Path 1 shows the fusion of membranes as a process involving an unstable fusion intermediate at b_1 and a stable fusion product at c_1 (other distinct intermediate structures may exist at points between a and b_1 but are omitted here for simplicity). All CM events fell into one of two categories. In the first category the CM event ended with all adjacent labeled membranes having the same amount of fluorescence. In the second case the amount of fluorescence which was transferred to the originally unlabeled ghosts was less than that in the originally labeled ghost after the pulse (Sowers, 1984). In the second case, the non-equilibrium

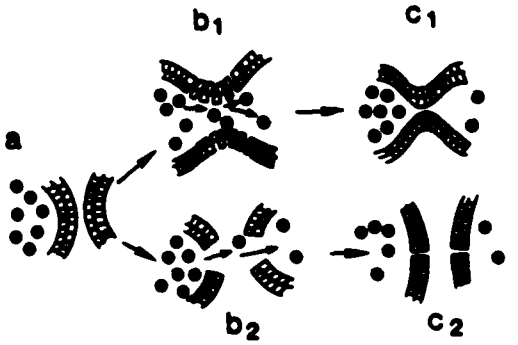

Fig. 3. Pathway by which an electric pulse leads to fusion-associated contents mixing events or nonfusion contents mixing events: a, unfused membranes in close contact (left compartment contains contents mixing indicator (solid circles); b_1, lumen at early stage of assembly with effective diameter greater than fluorescent indicator molecules; c_1 fusion product lumen with effective diameter less than fluorescnet indicator molecule (contents mixing and membrane fusion); b_2, movement of indicator molecules through concentric electropores (see text) to originally unlabeled compartment during pulse; c_2, electropores immediately after the pulse as resealed pores (contents mixing without fusion of membranes).

distributions in fluorescence was maintained until they could no longer be distinguished because of bleaching (about 3-5 mins depending on original level of labeling). In no case were non-equilibrium distributions perceived to approach equilibrium.

This indicates that the inside diameter of b_1 was not large enough or the lifetime of b_1 was not long enough to allow equilibrium in contents mixing to be achieved before the lumen diameter contracted (at c_1) to a diameter which is similar to or less than the diameter of the fluorescent soluble molecule. Such membrane mixing without contents mixing has been previously observed in plasma membranes of cultured cells fused with polyethylene glycol (Wojcieszyn et al., 1983) and could similarly be due to a submicroscopic lumen which is small compared to the probe molecule. This could also lead to the observed shortfall in FY_{cm+a} compared to FY_{mm} (Table I) seen at 60 mM (see above).

Path 2 (Fig. 3) shows at b_2 two concentric electropores, one in each membrane, which have a peak diameter large enough to permit significant passage of a contents mixing indicator to the originally unlabeled ghost. At c_2, the cessation of this passage is attributed to resealing of the electropore to be more consistent with the previous electropore literature, although new evidence indicates that the cessation of this passage may be due primarily to a cessation of electroosmosis (Sowers, 1988). Regardless of the diameter of pores at C_2, path 2 does not at any point provide an opportunity for the two originally separate membranes to ever come into contact with each other to form a fusion product. Although path 2 can be completely suppressed at S = 60 mM (Table I), paths 1 and 2 can be clearly distinguished from one another if dielectrophoresis is removed after the fusion-inducing pulse and visual inspection is used establish membrane-membrane attachment. It is possible that both pathways may occur simultaneously in the same pair of membranes in contact. From our previous study (Sowers, 1988) it appears that an increase in buffer strength from 20 mM to 60 mM has the effect of almost completely eliminating path 2 events while slightly increasing path 1 events (Fig 3 in Sowers, 1988 and Table I, this paper)

In contrast to the two pathways shown in Fig 3, Pilwat et al (1981), and later, in a variation, Dimitrov and Jain (1984), speculated that a set of two concentric electropores shared by the two membranes in close contact could be a membrane fusion intermediate. Incorporation of this feature into Fig. 3 is accomplished by considering the pathway a -> b_2 -> b_1 -> c_1. Although no experimental evidence has been presented to date to support that proposal, our data do not favor this pathway for the following reason. An increase in buffer strength slightly but clearly increases the probability of a MM event (Table I), which is represented by c_1 (Fig. 3), but decreases the passage of CM indicators through electropores (Sowers, 1986). Although the basis of this reduced passage is not understood, the decrease in CM indicator passage is most likely due to: i) a diminished population (numbers or sizes of electropores) at b_2, or ii) by the small inside diameters of the lumens (see above and Wojcieszyn et al., 1983). In the former case, the higher buffer strength would increase electrical conductivity and therefore more quickly discharge, or at least limit, the membrane hyperpolarization which was induced by the pulse. In the latter

case, fusion products which have lumens with reduced inside diameters would explain the discrepancy between FY_{cm+a} and FY_{mm} at 60 mM. From a kinetics point of view, a slight increase in c_1 fusion products caused by a buffer strength increase from 20 mM to 60 mM would not be likely from a large decrease in the population of precursor b_2 intermediates unless the step a -> b_2 was reversible and a buffer strength increase caused a large decrease in either the forward direction or a large increase in the reverse direction. In either case there must also be a corresponding but greater increase in either the step b_2 -> b_1 or the step b_1 -> c_1. Complexity argues against this hypothesis while simplicity argues in favor of the possibility that nonfusion contents mixing events are represented by the completely separate pathway: a -> b_2 -> c_2. Thus, electropores are not favored to be part of the fusion mechanism. The conclusion that electropores may not be involved in the fusion mechanism is also supported by the results of other studies using entirely different approaches (Sowers, 1987; Teissie & Rols, 1986). This suggests that electric field-induced membrane fusion may be due to a specific membrane structure or property which is induced simultaneously but independently of electropores.

REFERENCES

Ahkong, Q.F., and Lucy, J.A. (1986) Biochim. Biophys. Acta 858, 206-216.

Chernomordik, L.V., Sukharev, S.I., Popov, S.V., Pastushenko, V.F., Sokirko, A.V., Abidor, I.G., and Chizmadzhev, Y.A. (1987) Biochim. Biophys. Acta 902, 360-373.

Dimitrov, D., & Jain, R.K. (1984) Biochim. Biophys. Acta 779, 437-468.

Gingell, D. & Ginsberg, L. (1978) in Membrane Fusion (Poste, G. & Nicolson, G.L., Eds) Elsevier/N. Holland, Amsterdam. 791-833.

Knight, D.E., & Scrutton, M.C. (1986) Biochem. J. 234, 497-506.

Lucy, J.A., and Ahkong, Q.F. (1988) in Molecular Mechanisms of Membrane Fusion (Ohki, S., Doyle, Flanagan, T.D., Hui, S.-W., and Mayhew, E., eds) 163-179.

Pilwat, G., Richter, H.-P., and Zimmermann, U. (1981) FEBS Let 133, 169-174.

Pohl, H.A. (1978) Dielectrophoresis. Cambridge University Press, 579 pp.

Neumann, E., Schefer-Ridder, M., Wang, Y, and Hofschneider, P.H. (1982) EMBO J. 1, 841-845.

Schwister, K., and Deuticke, B., (1985) Biochim. Biophys. Acta 816, 332-348.

Serpersu, E.H., Kinosita, K., Jr., and Tsong, T.Y. (1985) Biochim. Biophys. Acta 812, 779-785.

Sowers, A.E. (1988) Biophys. J. 54, 619-626.

Sowers, A.E. (1986) J. Cell Biol. 102, 1358-1362.

Sowers, A. E. (1985) Biophysical J. 47, 519-525.

Sowers, A.E. (1984) J. Cell Biol. 99, 1989-1996.

Tsong, T.Y. (1983) Biosci. Rept. 3,487-505.

Wojcieszyn, J.W., Schlegel, R.A., Lumley-Sapanski, K, & Jacobson, K.A. (1983) J. Cell Biol. 96,151-159.

Table I. Fusion yields, in percent, from contents mixing events (FY_{cm}), from contents mixing events in which membrane-membrane attachement could be demonstrated (FY_{cm+a}), and from membrane mixing events (FY_{mm}) as influenced by pulse field strength, E (in V/mm), pulse decay half-time, $T_{1/2}$ (in msec), and buffer strength, S (in mM).

Conclusion: Event yield was always proportional to pulse decay half-time and electric field strength regardless of event indicator (Table I) and was consistent with previous findings (Sowers, 1988). An increase in buffer strength from 20 to 60 mM always caused a large decrease in FY_{cm}, a small to moderate decrease in FY_{cm+a}, and a moderate increase in FY_{mm}. At 20 mM, FY_{cm+a} essentially agrees, within experimental error, with FY_{mm}.

Donor	E	$T_{1/2}$	S	FY_{cm}	FY_{cm+a}	FY_{mm}
B	500	0.7	20	57	38	36
			60	34	34	49
C	500	0.9	20	60	38	33
			60	28	27	54
A	600	0.5	20	20	6	7
			60	0	--	15
A	600	0.7	20	37	26	22
			60	14	14	37
B	600	0.9	20	67	62	58
			60	*	--	*
A	700	0.5	20	25	17	17
			60	9	9	31
C	700	0.7	20	80	74	58
			60	*	--	*
C	700	0.9	20	92	92	72
			60	*	--	*
B	800	0.5	20	60	45	34
			60	16	15	50

* - extensive fragmentation of ghosts into numerous vesicles without fusion. Note: All FYs measured with a pulse of E = 500 V/mm at $T_{1/2}$ = 0.5 msec were < 2 %.

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